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July 28, 2006

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APPLICATION NUMBER: 60/504,530

FILING DATE: September 19, 2003

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS US60/504,530

**By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office**



L. Edelen

**L. EDELEN
Certifying Officer**

16569 U.S. PTO
09/19/03

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL983811750US

INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname		Residence (City and either State or Foreign Country)		
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<input checked="" type="checkbox"/> Additional inventors are being named on the 1 separately numbered sheet attached hereto					
TITLE OF THE INVENTION (280 characters max)					
DIAGNOSTIC SYSTEM FOR DETECTING OTOLARYNGOLOGIC PATHOGENS AND PATHOGENS IN OTHER ARENAS					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number				Place Customer Number Bar Code Label here	
OR Type Customer Number here					
<input checked="" type="checkbox"/> Firm or Individual Name		Nixon Peabody LLP			
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification		Number of Pages	39	<input type="checkbox"/> CD(s), Number	
<input type="checkbox"/> Drawing(s)		Number of Sheets		<input type="checkbox"/> Other (specify)	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE	
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees				AMOUNT (\$)	
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge fees which may be required, or credit any overpayment to Deposit Account Number:				14-1138	
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.				\$80.00	
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:					

Respectfully submitted,

Date 9/19/03

SIGNATURE Edwin V. Merkel

REGISTRATION NO.
(if appropriate)

40,087

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Docket Number:

176/61640 (6-
1258)

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

R713814.1

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R713814.1

FEE TRANSMITTAL FOR FY 2003

Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ 80)

Complete if Known

Application Number

Filing Date

First Named Inventor

Miller et al.

Examiner Name

Art Unit

Attorney Docket No.

176/61640 (6-1258)

METHOD OF PAYMENT (check all that apply)

☒ Check ☐ Credit Card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account:

Deposit
Account
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14-1138

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The Commissioner is authorized to: (check all that apply)

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Fee (\$)	Small Entity Fee Code	Fee (\$)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) (\$ 80)

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Extra Claims	Fee from below	Fee Paid
	-20** =	X	= 0
Independent Claims	-3** =	X	= 0
Multiple Dependent	X	=	= 0

Large Entity Fee Code	Fee (\$)	Small Entity Fee Code	Fee (\$)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$ 0)

**or number previously paid, if greater. For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Small Entity

Fee Code	Fee (\$)	Fee Code	Fee (\$)	Fee Description
1051	130	2051	65	Surcharge - late filing fee or oath
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet
1053	130	2053	130	Non-English specification
1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action
1251	110	2251	55	Extension for reply within first month
1252	410	2252	205	Extension for reply within second month
1253	930	2253	465	Extension for reply within third month
1254	1,450	2254	725	Extension for reply within fourth month
1255	1,970	2255	985	Extension for reply within fifth month
1401	320	2401	160	Notice of Appeal
1402	320	2402	160	Filing a brief in support of an appeal
1403	280	2403	140	Request for oral hearing
1451	1,510	1451	1,510	Petition to institute a public use proceeding
1452	110	2452	55	Petition to revive - unavoidable
1453	1,300	2453	650	Petition to revive - unintentional
1501	1,300	2501	650	Utility issue fee (or reissue)
1502	470	2502	235	Design issue fee
1503	630	2503	315	Plant issue fee
1460	130	1460	130	Petitions to the Commissioner
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)
1806	180	1806	180	Submission of Information Disclosure Stmt
8021	40	8021	40	Recording each patent assignment per property (times number of properties)
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))
1801	750	2801	375	Request for Continued Examination (RCE)
1802	900	1802	900	Request for expedited examination of a design application

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$ 0)

CERTIFICATE OF MAILING OR TRANSMISSION [37 CFR 1.8(a)]

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Date

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SUBMITTED BY

Name (Print/Type)

Edwin V. Merkel

Registration No.
(Attorney/Agent)

40,087

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Signature

Edwin V. Merkel

Date

Sept. 19, 2003

SEND TO: Commissioner for Patents
P.O. Box 1450
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R713815.1

EXPRESS MAIL CERTIFICATE

DOCKET NO.: 176/61640 (6-1258)

APPLICANTS: Benjamin L. Miller, Scott R. Horner, Farhan Taghizadeh, and
Lewis J. Rothberg

TITLE: DIAGNOSTIC SYSTEM FOR DETECTING
OTOLARYNGOLOGIC PATHOGENS AND PATHOGENS IN
OTHER ARENAS

Certificate is attached to the **Provisional Application for Patent Cover Sheet
and Fee Transmittal (3 pages)** of the above-identified application.

"EXPRESS MAIL" NUMBER: EL983811750US
DATE OF DEPOSIT: September 19, 2003

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Shawn A. Lockett
(Typed or Printed Name of Person Mailing
Paper or Fee)


(Signature of Person Mailing Paper or Fee)

EXPRESS MAIL CERTIFICATE

DOCKET NO.: 176/61640 (6-1258)

APPLICANTS: Benjamin L. Miller, Scott R. Horner, Farhan Taghizadeh, and
Lewis J. Rothberg

TITLE: DIAGNOSTIC SYSTEM FOR DETECTING
OTOLARYNGOLOGIC PATHOGENS AND PATHOGENS IN
OTHER ARENAS

Certificate is attached to the **Provisional Patent Application (39 pages)** of
the above-identified application.

"EXPRESS MAIL" NUMBER: EL983811750US
DATE OF DEPOSIT: September 19, 2003

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Shawn A. Lockett
(Typed or Printed Name of Person Mailing
Paper or Fee)


(Signature of Person Mailing Paper or Fee)

TITLE: **DIAGNOSTIC SYSTEM FOR DETECTING
OTOLARYNGOLOGIC PATHOGENS AND PATHOGENS
IN OTHER ARENAS**

INVENTORS: **Benjamin L. Miller,
Scott R. Horner,
Farhan Taghizadeh, and
Lewis J. Rothberg**

DOCKET NO.: **176/61640**

PROVISIONAL PATENT APPLICATION

R714227.1

DIAGNOSTIC SYSTEM FOR DETECTING OTOLARYNGOLOGIC PATHOGENS AND PATHOGENS IN OTHER ARENAS

INTRODUCTION:

Current methods of diagnosing bacterial, viral, and fungal infections of the ear, nose, throat, and upper respiratory tract rely on clinical findings or culture samples processed through a clinical laboratory. These methods are suboptimal because they introduce a delay between the point of care and the exact diagnosis of the patient's ailment. Subsequently, there is an increase in the cost of care, overuse of antibiotics leading to worsening resistance patterns, and potentially dangers of missing new, dangerous respiratory infections.

In addition to detecting disease causing pathogens, these devices could also be used in the diagnoses of certain disease states that do not result from infection. The detection of certain markers indicative of physical abnormalities that manifest themselves as a disease state (ie. acid reflux disease, genetic abnormalities) is also possible and a likely extension of this technology.

The present invention is meant to broaden the capabilities for point-of-care infection detection, allowing for the rapid diagnosis of many common bacterial, viral, and fungal infections.

The present invention also extends the scope of use of the method of reflective interferometry, disclosed previously. Many of the concepts described herein will likely also be applicable to other sensor platforms (porous silicon, etc.).

DESCRIPTION OF THE INVENTION:

The present invention consists of the combination of a probe chip (itself consisting of a probe molecule or series of molecules attached to an appropriately prepared reflective surface via a covalent or non-covalent attachment), a reader device, and the use of this combined system in the diagnosis of ENT (ear-nose-throat) related infections. ENT infections include, but are not limited to, middle ear infections, laryngeal infections, sinusitis, and throat infections. The specific organisms we intend to target and identify with our ENT suite of chips include, but are not limited to common anaerobes, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, α and β Hemolytic *Streptococcus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, viruses like parainfluenzae, influenzae, and rhinovirus, and any host of fungi and parasites contributing to diseases of the ear nose and throat.

These devices are not limited to ENT related diseases and have potential applications in many other areas. We envision extending this technology to include "organ specific" disease detection, which would consist of a chip designed for a specific disease state, and not explicitly a single organism. A few examples of these include, but are not limited to: Respiratory chips that detect pneumonia, bronchitis, and other pulmonary ailments from any host of viral, fungal, and bacterial pathogens. Gastrointestinal (GI) chips that can detect the presence of organisms causing diseases like ulcers, gastroenteritis, and small and large bowel infections from any host of bacterial, fungal, viral, and parasitic organisms. Wound chips that detect the presence of infections in wounds, including infections from implanted medical devices. Blood chips (sepsis chips) that detect the presence of bacteria, viruses, fungi, and parasites in blood. Neurologically focused chips that can be used to detect the presence of bacteria, viruses, and fungi in

cerebrospinal fluid. Genitourinary chips that focus on a wide range of infections from urinary tract infections to sexually transmitted disease. General surveillance chips implanted in devices like respirators or used in health institutions to carry forth inspection of organisms common to nosocomial infections.

The following examples detail some of the specific molecular targets we intend to detect in order to diagnose the given disease state.

EXAMPLES:

Example 1. Chip functionalized with DNA probe sequences for detecting rRNA in bacteria, viruses, fungi, and parasites. The target sequences are not necessarily limited to rRNA.

- a. The arraying of multiple probes on a single chip for point of care detection. These probes can be for organ-specific disease combinations (like a chip for all sinus infections), combining probes for bacteria, viruses, or fungi. They can also be for disease specific combinations (URI viral chip, bacterial pharyngitis chip, fungal otitis chip), etc.
- b. The placement of single probes on chips for rapid point of care detection. An example would be a new rapid streptococcus point of care chip.
- c. Attached is a manuscript for research outlining this technique as well as a presentation summarizing its use.

Example two: Antibody-functionalized chip for detection of bacteria, viruses, fungi, or any host of allergic diseases. These antibodies would be raised towards specific protein, peptide, or small molecule targets, unique to the organism or disease of interest like allergic rhinitis. Patient serum or secretions can be placed on these chips. The diagnosis would be generated using these antibody mobilized chips.

Example three: DNA or antibody chips used for rapid molecular detection of cellular morphology. These biomarker chips allow for rapid detection of cellular features, as in determining prognostic factors for cancer behavior. Examples of such biomarkers include, but are not limited to p53, Bcl-2, Cyclin D1, c-myc, p21ras, c-erb B2, and CK-19.

Example four: Hyaluronic acid disaccharide chip. Polysaccharide functionalized chip for the detection of *Streptococcus pneumoniae* hyaluronate lyase. Simple chip that identifies the presence of the most common etiologic agent responsible for AOM (acute otitis media) and for invasive bacterial infections in children of all age groups.

Example five: Pepsin activity detection chip. Protein or peptide functionalized chip that indicates the presence of pepsin through the inherent enzymatic activity and in turn identifies possible acid reflux disease (GERD). This is enabled through the use of proteins or peptides that are the normal substrates of pepsin enzymatic activity

Example six: A chip designed to rapidly detect molecules like B-2 transferrin that are sensitive to the diagnosis of cerebrospinal fluid leaks. These chips may use any range of protein detection techniques to detect the presence of this molecule in patient sinus or ear specimens.

Example 7: Lipopolysaccharide A (LPS) detection chip. Immobilize molecules on the surface of the chip that are sensitive and specific for the molecule LPS, the causative agent behind most cases of sepsis.

Method of use: The chips would be stored in the physician's office, hospital, or operating room suite, wherever point of care detection is most convenient for the physician or other health care practitioner. These chips can also be used by clinical laboratories to make more accurate, more rapid detection.

For infectious diseases, there are three usual methods for sample collection in the diseased organ system. First, upon suspicion of an infectious disease etiology, the infection site can be swabbed as per usual protocol for obtaining cultures for microbiological processing. The practitioner may or may not see clinical evidence of the infection. Given the chip sensitivity, an area can be swabbed if the practitioner has the mere suspicion of infection. Second, for other diseases like sinusitis or urinary tract infections, the patient may produce a sample (sputum, urine, etc) that can be collected for chip evaluation. Third, for diseases like sepsis or meningitis, appropriate serum or CSF can be collected by a licensed practitioner and placed on the chip.

For other categories of diagnostic detection not related to infectious etiologies, similar techniques will be employed to obtain a patient sample and place it on the chip for functionalization and detection.

Once the sample is collected, it will be placed on the appropriate chip for diagnosis. As noted above, the chip may be designed per disease organ, per infectious etiology, as a single organisms detection tool, or for any group of relevant molecules necessitating detection. Once the sample is placed on the chip, it will be processed potentially through a series of simple washes. We anticipate that with continued technology development, multiple washes will not be needed. The chip will then be scanned in the examination setting. This detection device will use a laser to first scan the surface of the chip. On multiple probe chips, there will be a recorded map of the probes such that specific target binding can be assessed. The laser will reflect onto a photodiode, and a computer processor will determine positive binding based on previous set algorithms.

The scanned chip data will translate into a simple report of infectious etiology for the physician/health practitioner to evaluate. This data can then be used to determine treatment options for the patient.

One alternative technique for this device will be a delayed evaluation after the swabbed sample is incubated for several hours and then wiped onto the chip. This will still allow for point of care detection, or it may be an alternative to current clinical laboratory organism evaluation techniques.

Example of Biosensor Design Strategy for *Pseudomonas Aeruginosa* in Otolaryngology

Problem Addressed: The ability to detect bacterial and viral infections at the point of care would significantly impact Otolaryngology practice, improving diagnostic accuracy and reducing unnecessary antibiotic use. In this study, we set forth a design for a *Pseudomonas Aeruginosa* Biosensor.

Methods and Measures: Silicon wafers carrying a 150 nm layer of thermal oxide were derivatized so as to provide an immobilized layer of streptavidin. Biotinylated DNA sequences complementary to *Pseudomonas*-specific rDNA were attached to the streptavidin surface. Complementary synthetic DNA sequences were hybridized to the probes to verify probe immobilization and specificity. Treatment of analogously functionalized chips with *Pseudomonas* strain PAO-1 cultures was used to test the suitability of the technique for detection of this bacterium. Surface analysis was conducted using reflective interferometry to detect surface thickness changes consistent with target hybridization to probe.

Results: We successfully detected complementary DNA sequence binding to the biosensor surface using reflective interferometry. The *Pseudomonas* probes bound both centrifuged and freshly cultured stocks of *Pseudomonas* in LB media. The probes did not bind similarly prepared stocks of *E. coli*. Serial dilution experiments indicated a photo-detection capability of 125-160 organisms per 5 µl spot

Conclusions: Biosensors represent a new frontier in Otolaryngology, with the potential to significantly expand diagnostic capabilities at the point of care. We used one specific biosensor design and demonstrated its utility in detecting *Pseudomonas aeruginosa* from pure cultured stocks using reflective interferometry, a simple, label-free detection method.

Clinical Significance of Study: This study demonstrates the ability to use silicon oxide coated chips and reflective interferometry to detect bacteria in vitro, setting the stage for future development and clinical use in otolaryngology.

Introduction

Point of care diagnosis of infectious organisms would dramatically change treatment paradigms in otolaryngologic disease. For example, the prevalent spread of bacterial antibiotic resistance could be slowed if better diagnostic capabilities existed at the point of care. Additionally, such testing capabilities could reduce the cost of care, better enabling the correlation of symptoms and clinical findings to the presence of infectious organisms. Such point of care technologies are widespread in modern medical care, from blood glucose measurements to rapid *Group A Streptococcus* testing. Acceptability of basic rapid testing as well as its many benefits has prompted research to find wider uses for this technology in otolaryngology.

Bacterial and viral species identification using comparative analysis of rDNA sequences is a well established method of bacterial identification.ⁱⁱ Recent advances in targeting ribosomal nucleic acid sequences (rRNA) with DNA (rDNA) probes represents an attractive technique for rapid detection without sequence amplification, given the abundance of such ribosomes in bacteria.^{iii iv} Using sequence databases, bacteria specific sequences have been identified, with sequences for *Pseudomonas* proving reasonably sensitive for detection.^v *Pseudomonas aeruginosa* represents an excellent organism for early biosensor development in otolaryngology not only because of its pathogenicity in ear infections like otitis externa, but also because of its presence in normal ears.^{vi} Detection research must be geared towards providing accurate counts of such organisms in the clinical setting.

As part of a program targeting the development of simple, label-free sensors for rapid clinical and home diagnostic use, we recently developed reflective interferometry as a new platform technology. This technique, a close relative of ellipsometry, allows detection of a very small change in the coating thickness of a reflective surface (such as silicon) when new molecules bind the surface. Using polarized light, this thickness change can be seen with changes in reflected incident light intensity.^{vii} By modifying the surface of the silicon chip with probe molecules, such as DNA, antibodies, or even small molecules,^{viii} for a target pathogen, specific detection of that pathogen is possible.

The ability of this technique to bind DNA and its complementary sequences has been demonstrated. The goal of this study was to test the utility of reflective interferometry for the detection of *Pseudomonas aeruginosa* samples. Chips were prepared using known DNA sequences specific for *Pseudomonas* ribosomal RNA (rRNA). Furthermore, the sensitivity of the technique, as well as its specificity, was evaluated. The eventual goal of this research is to take such chips into the clinical arena and perform point of care diagnostic testing specific for otolaryngology.

Materials and Methods

Surface Chemistry

Silicon oxide wafers 6" diameter bearing a layer of 150 nm thick thermal oxide were obtained and sent to a commercial vender (Xerox Corporation, Rochester NY) for thermal oxide coating. These wafers were cut into 2.5 x 2.5 cm square chips. Care was taken to avoid scratching or otherwise marring the chip surface during all processing steps. All reagents (with the exception of DNA sequences, *vide infra*) were purchased from Sigma-Aldrich (St. Louis, Missouri). The chips were soaked in piranha etch solution (9 ml 3% H₂O₂ in 21 ml of 96% H₂SO₄) for 30 minutes. The chips were rinsed with ddH₂O and dried under a stream of nitrogen gas. The chips were then silanized with a 5% 3-aminopropyltriethoxysilane solution 5 % in acetone (96% reagent grade) for 1.5 hours. The chips were rinsed with ddH₂O and dried under a stream of nitrogen gas. After baking the silanized chips at 100 degrees C for 1 hour, they were then treated with a solution of 2.5 % Glutaraldehyde in 50 mM PBS (pH 7.4) for 45 minutes. The chips were rinsed with ddH₂O and dried under a stream of nitrogen gas. Each resulting glutaraldehyde-functionalized chip was then coated with 500 µl of streptavidin (0.05 mg/ml in

PBS pH 7-7.5) for 45 minutes. The chips were rinsed with ddH₂O and dried under a stream of nitrogen gas. At this point, the chips were ready for the immobilization of the biotinylated DNA probes.

Probes

The well-studied streptavidin-biotin interaction^{ix} was utilized to bind the DNA probes to the chip surface. Two biotinylated probes for *Pseudomonas* were purchased from a commercial supplier (Invitrogen Life Technologies, Carlsbad, California). and used throughout this study:

Probe 1 5'-Biotin-CCT-TGC-GCT-ATC-AGA-TGA-GCC-TAG-GT-3' ^x
 Probe 2 5'-Biotin-CTG-AAT-CCA-GGA-GCA-3' ^{xi}

The biotinylated DNA probes were brought up to a concentration of .05 micromole/ml in PBS (pH 7.5). 5 µl of this solution was pipetted on the chips at each desired spot, and allowed to stand in a high-humidity chamber for 45 minutes. Chips were then rinsed with 50 mM PBS, followed by dd H₂O. The chips were now ready for treatment with either solutions of synthetic, complementary DNA, or with bacteria. Figure 1 gives a basic schematic of the chip functionalization process. .

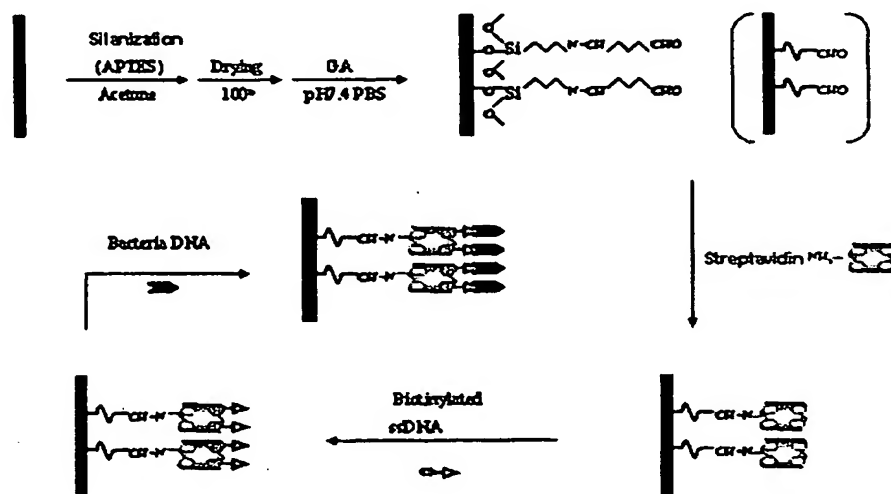


Figure 1: Schematic diagram for the chemical coating of the biosensor.

Probe Testing

Single stranded DNA sequences to Probe 1 and Probe 2 were purchased from a commercial supplier (Invitrogen Life Technologies, Carlsbad, California), and diluted to a

concentration of 0.05 micromole/ml in PBS. Each prepared chip's shape was traced onto graph paper, to mark the position placement of the probe and the subsequent complementary target sequence. The chips were prepared such that four spots were placed on the chip, with two having just placement of Probe 1 and two with only Probe 2. Once the Probes had been placed, the chips were washed with dd H₂O and dried under a stream of nitrogen gas. The chips were then immediately assessed for probe binding to the chip.

Bacterial Processing Technique and Counts

Standard microbiology handling techniques were used to plate colonies and bring up culture solutions in LB media. The PAO-1 strain of *Pseudomonas aeruginosa* and the JM109 strain of *E. Coli* were obtained from the Department of Microbiology at Strong Memorial Hospital. Several colonies were swabbed from the culture plate into approximately 7-10 cc of LB media and cultured for 12 hours prior to experimentation. In the first set of experiments, 500 µl of cultured media was centrifuged at 12,000 x G for 10 minutes. The pelleted cells were resuspended in 1 ml of 50 mM PBS (pH 7-7.5). In the first set of bacterial experiments, this solution was diluted 1:5 in PBS. For the second set of experiments, the bacteria were taken directly out of the liquid LB media after culture for chip experimentation. In the final serial dilution experiment, overnight cultures were taken and diluted in 0.9% NaCl in sequential 1/10 dilutions. Each dilution was then plated on LB agar plates in sets of 3, and the plates with 30-300 colonies were counted, with averages being obtained for the set dilution. Standard solution counts based on these dilutions were obtained using standard microbiology protocols for this procedure^{xiii}.

Chip Bacterial Coating

Each chip was placed on grid paper, and the coordinates of the probes were marked. For each experiment, 5 µl of the bacterial preparation was placed on the coordinates of the probe and hybridized for 45 minutes at room temperature, followed by either a dd H₂O wash or a PBS wash and then nitrogen gas drying. To prevent spot drying, hybridization occurred in closed petri dishes with water soaked cotton balls to maintain moisture.

In the first set of bacteria experiments, the concentrated *Pseudomonas* and *E. coli* in 1:1 and 1:5 dilutions of PBS were spotted onto the *Pseudomonas* probes. The *E. coli* served as the control bacteria for each set of experiments. In the second set of experiments, 5 µl of fresh bacteria was taken from the LB media, and spotted on the *Pseudomonas* probes. Again, *E. coli* served as the control organism. LB media alone was also used as a control. In the last set of experiments, dilutions of *Pseudomonas* and *E. coli* in 0.9% NaCl were placed on the chips. These same dilutions were plated onto LB agarose plates for the counts. These chips were optically scanned to determine the detection limit for spot detection.

Reflective Interferometry

All chips were processed by a single investigator in an established optics laboratory at the University of Rochester. The probe light for detection is derived from a 450 Watt Xe lamp monochromatized to approximately 1 nm bandwidth using a spectrometer. The light is guided through two apertures approximately 5 mm in diameter and separated by 60 mm to enforce collimation to better than .5 degrees. The beam is incident on the chip surface at 70.6 degrees, which is the reflectivity minimum. The light is brought through several polarizers. The reflected light is observed onto a Princeton Instruments (Monmouth, NJ) CCD camera without imaging optics (Figure 2). The theory, technique and algorithm for obtaining a computerized surface map of this chip will be described in detail elsewhere.^{xiii} In short, the peak intensity of the spots were compared to the background. The intensity of the peaks in the computer processed image are relative to the background intensities of non-spotted parts of the chip, and software automatically re-scales all the data for each chip. In this paper, the three dimensional X,Y,Z contour images and the one dimensional, X, Y axis side-view of the three dimensional picture are shown for purposes of clarity.

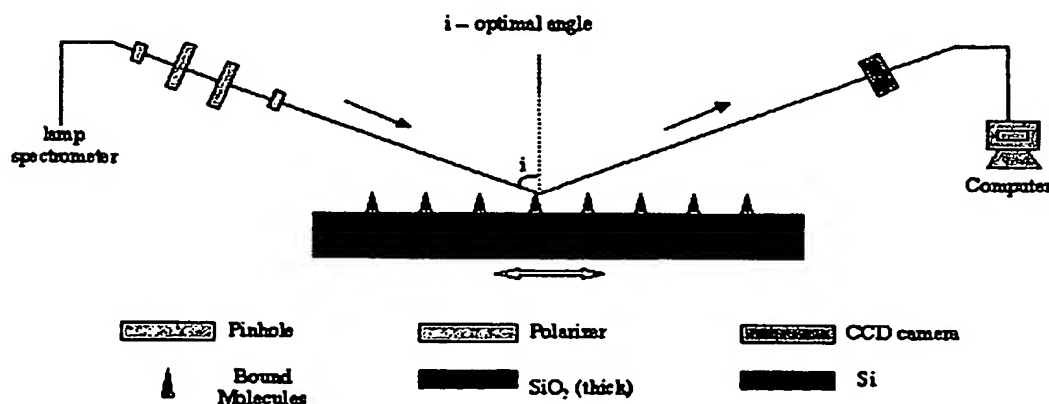


Figure 2 Schematic for the reflective interferometry laboratory set-up. The i (optimal value) is 70.6 degrees. The camera records the light and a computer algorithm produces an image comparing spots to background.

Results

Probe Binding Chip Experiments

Probes 1 and 2 for *Pseudomonas* were optically evaluated to assess their binding to the chip surface. The peak intensities were evaluated to assess visualization of this probe on the chip surface. Figure 3 and Figure 4 demonstrates the ability of the optical detection to see our selected probes on the chip surface.

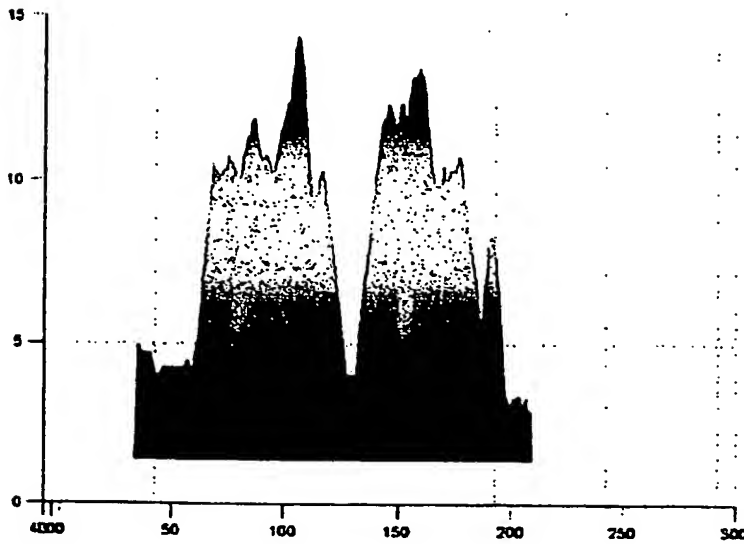


Figure 3- Optical scanning of Probe 1 (right) The X axis represents a relative scale for distance along the chip surface, while the Y axis represents relative peak intensity. The peaks show distinct binding of the probes to the chip surface.

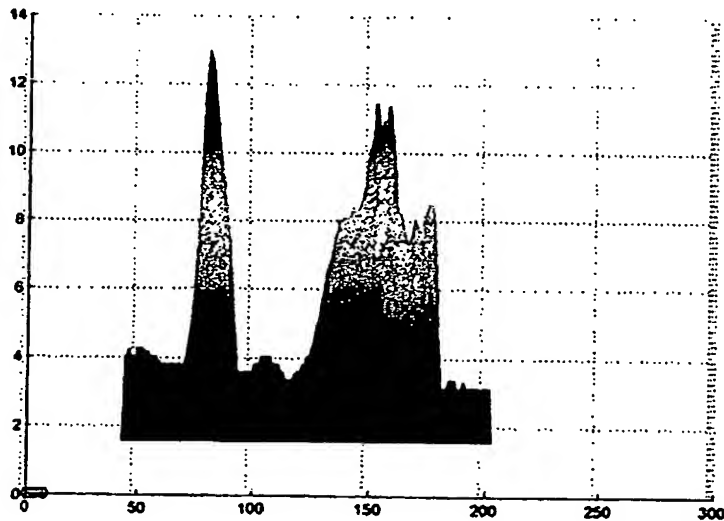


Figure 4- Optical scanning of Probe 2 (right) The X axis represents a relative scale for distance along the chip surface, while the Y axis represents relative peak intensity. The peaks show distinct binding of the probes to the chip surface

R714227.1

Experiment 1, Concentrated Bacteria

Following treatment with concentrated solutions of bacteria, the spots were immediately visible with the naked eye, without optical scanning (Figure 3). This "naked-eye" detection is likely due to light scattering off the surface of the chip. After optically scanning the chips, large peaks were noted for both the 1:1 and the 1:5 dilutions of the concentrated *Pseudomonas* organisms after both the dd H₂O and PBS rinse, while the *E. coli* spots did not demonstrate comparable intensity peaks over background. The PBS rinse provides an obvious visual display of a "darker" spot, and this is reflected in the optical peak intensities. As described above, the current scanning technique and visualization algorithm makes a comparative display of the darkest spot on the chip to the background, and displays the relative intensities for that specific chip. One also sees some salt streaking on the PBS rinsed chips after they are dried. The streak intensities were well below the spot intensities for these chips.

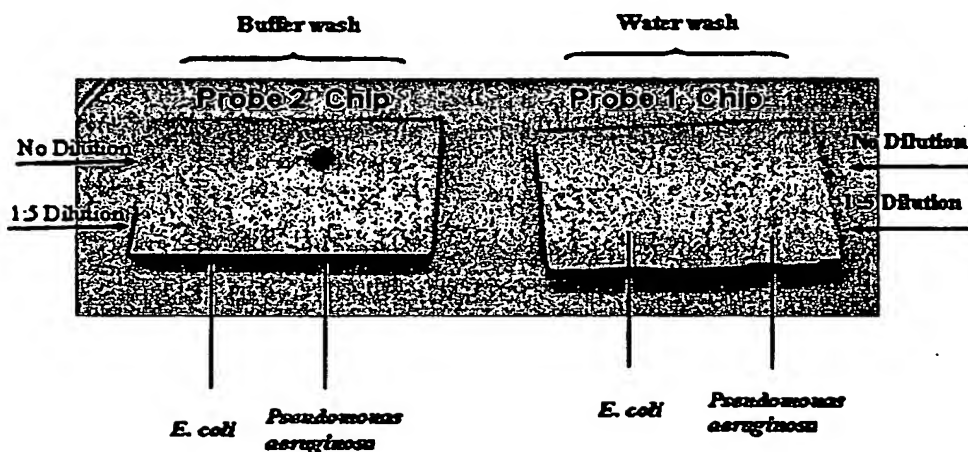


Figure 5 Four probe spots were placed on each chip: one chip for Probe 1 and one for Probe 2. Concentrated bacteria was resuspended in 1ml (1:1) or 5 ml (1:5) PBS. The Probe 2 chip was rinsed with PBS, while the Probe 1 chip with dd H₂O. Sufficient bacteria remained on the probe 1 chip to allow naked-eye detection of bacteria following PBS rinse.

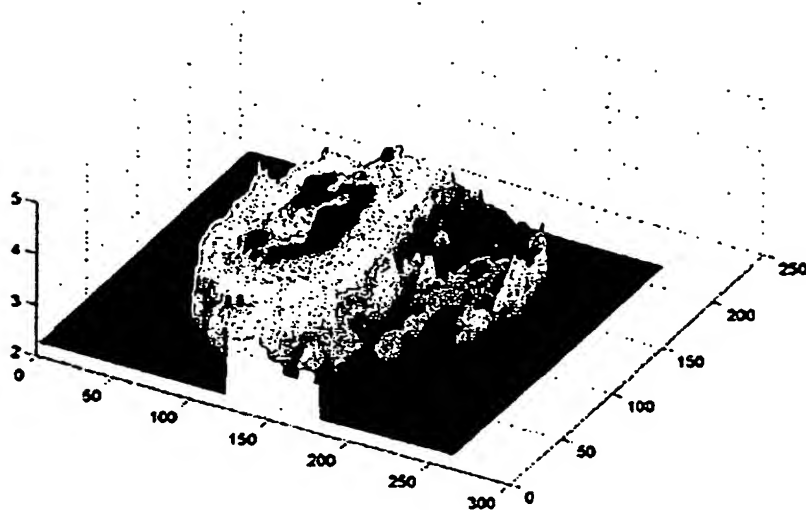
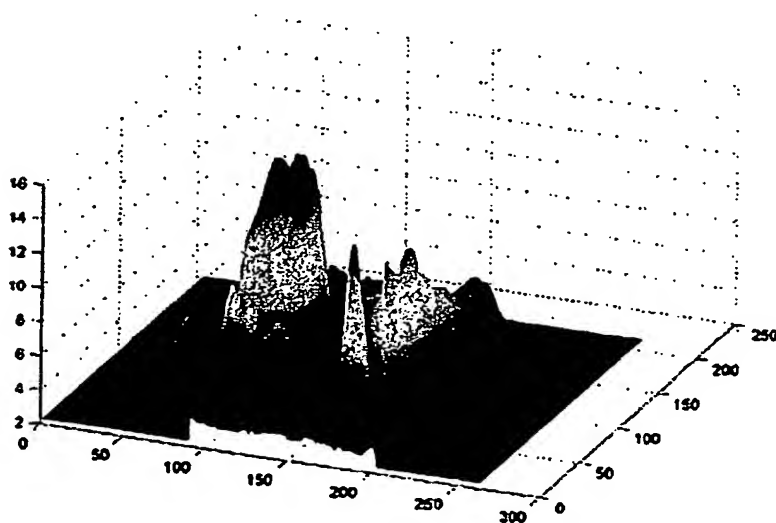


Figure 6 Scanned surface over *E. coli* section of Probel chip, which was rinsed with dd H₂O after hybridization. The X and Z axes are relative distances on the chip surface, while the Y axis represents the intensities. The small peaks likely represent attached probe on the surface and some salt residue.



R714227.1

Figure 7

Scanned surface over *Pseudomonas* section of Probe1 chip, which was rinsed with dd H₂O after hybridization. The X and Z axes are relative distances on the chip surface, while the Y axis represents the relative intensities. The large peak on the left demonstrates 1:1 concentrated solution and the right peak shows a 1:5 diluted solution of the bacterial pellet in PBS. . The peak on the right may be part of the other spot, but is more likely an artifact due to dust on the surface of the chip.

Experiment 2, Fresh Bacteria

Four spots were placed on each chip, the top two with Probe 1 for *Pseudomonas* and the bottom 2 with Probe 2 for *Pseudomonas*. On each pair of two spots, fresh LB and fresh LB with cultured bacteria were placed on the probes. No recognizable peaks were noted for the control LB media alone. There were distinct peaks for the *Pseudomonas* in LB, and there were no peaks noted for *E. coli* in LB. The results for Probe 1 and Probe 2 were similar. All chips in this experiment were rinsed with PBS after hybridization to the probe.

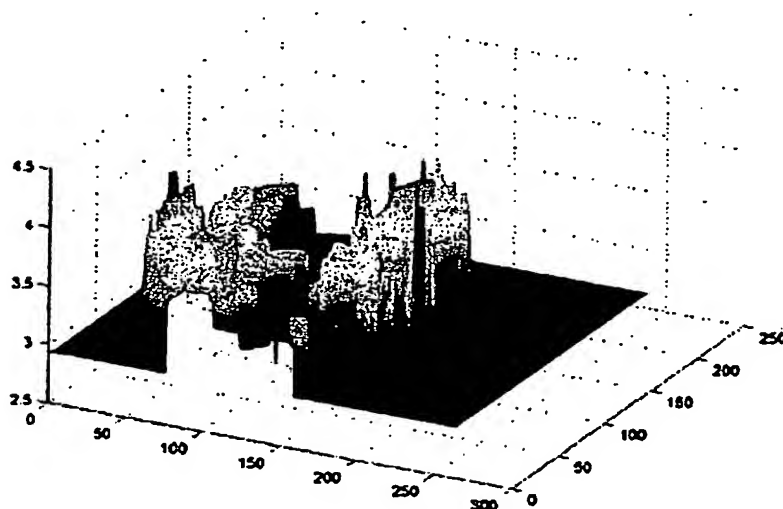


Figure 8 Scanned surface of two spots for Probe 1 chip. The left side had fresh LB placed on Probe 1, while the right side had *E. coli* in fresh LB placed for hybridization. The peak intensities of *E. coli* are minimally different than the LB control. Again, The X and Z axes are relative distances on the chip surface, while the Y axis represents the intensities.

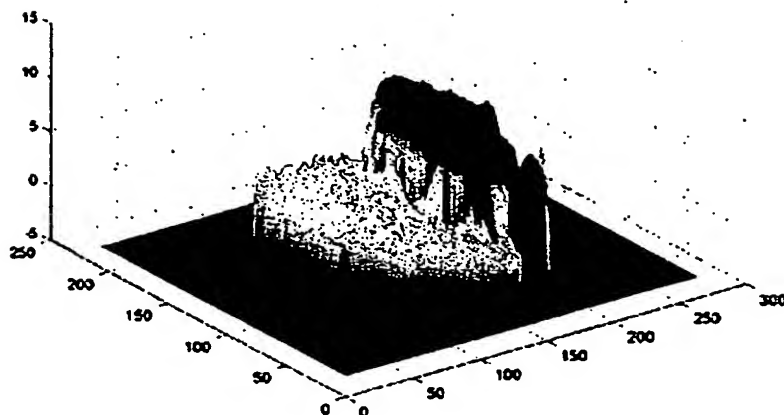


Figure 9 Scanned surface of two spots for Probe 2 chip. The left side had fresh LB placed on Probe 2, while the right side had *Pseudomonas* in fresh LB placed for hybridization. The peak intensities for this were very significant. Again, the X and Z axes are relative distances on the chip surface, while the Y axis represents the intensities. Similar results occurred for this experiment using Probe 1.

Experiment 3, Bacterial Counts

The bacteria were diluted in 0.9% NaCl and spotted from this solution. These same dilutions were plated in sets of three, with hand counted colony averages of 30-300 being used for final counts. In the first set of bacterial counts, 2.49×10^7 Colony Forming Units (CFU) of *Pseudomonas* were in each ml of solution. The dilution at which the peaks were no longer visible was 1/100,000 (Image 9), yielding a maximum optical detection of 24,900 CFU/ml of solution. The cut-off dilution was the same for chips using both Probe 1 and Probe 2. Since each spot consisted of only 5 μ l of solution, the limit of detection was 125 CFU/spot detection. Repetition of this experiment was completed with limits of 160 CFU/ 5 μ l spot being detected.

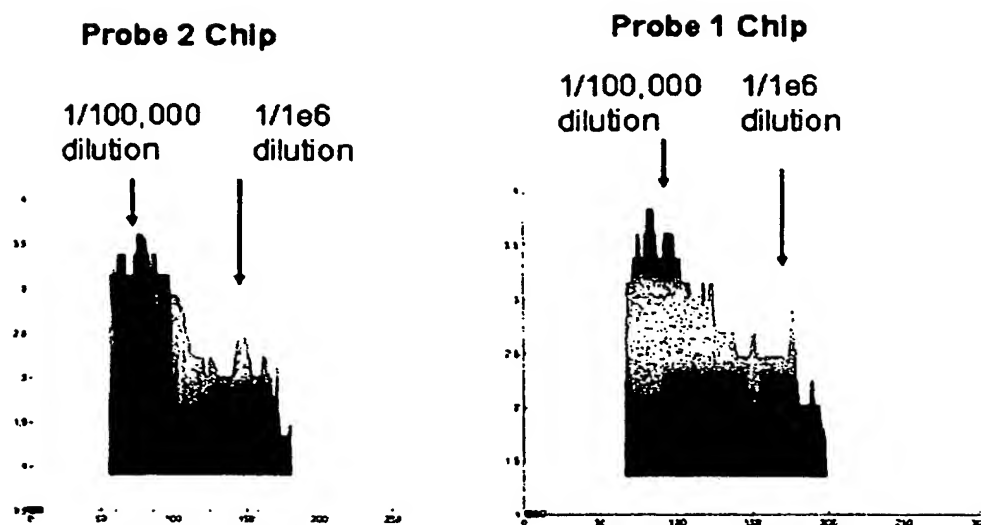


Figure 10 Two dimensional optical images of scanned chips for Probe 2 (left) and Probe 1 (right). The cut off dilutions of 1/100,000 is evident, as peaks are noted for this dilution and do not exist for the $1/1 \times 10^6$ dilution. Again, the X axis represents relative distance on the chip, and the Y axis represents peak intensity.

Discussion

Point of care testing has many clinical advantages for physicians and patients. Besides convenience, the ability to better diagnose disease at the point of care improves overall quality and helps reduce costs. While there are relatively few available tests, there is excellent data that such testing positively impacts care.^{xiv} In the field of otolaryngologic infectious disease, there is active interest in furthering point of care infectious testing. Examples include research in evaluating new rapid *Group A Streptococcus* tests^{xv} as well as advances in development of the electronic nose.^{xvi} Our group embarked on this study to advance new point of care technologies, and focus their use in future diagnosis of otolaryngologic infections.

Molecular detection and genotyping of pathogens is providing more rapid and accurate basis for laboratory detection.^{xvii} Using small DNA probes targeting rRNA sequences potentially allows organism detection without sequence amplification, given the vast amounts of rRNA in cells. With highly sensitive and selective probes, complementary rRNA sequences should be available in dividing bacterial cultures with short generation times in proper media, like *Pseudomonas aeruginosa*. Given the established sensitivity of our chosen probes, we hypothesized that the bacterial rRNA could be easily targeted without active steps to isolate or amplify these sequences.

The ability to bind DNA sequences to silicon oxide chips and perform rapid hybridizations on this surface represents a good basis for faster detection technologies. In

accordance with the selected sensor device, this form of reflective interferometry using a white light source (Xe lamp) allows for a detailed view of the chip surface, especially when detecting small molecules or small concentrations of bacteria.

The ability to detect organisms fresh out of cultured media represents a big step in reducing specimen processing steps. The media did not impact chip processing. Furthermore, the ability to dilute these organisms from the media allowed for the count experiments. The ability to get distinct "cut-off" peak intensities from these serial dilutions provides us with an excellent tool for comparing future variations in chip manufacturing, their long-term storage, various rinses, and new optical detection techniques. It also allows us to perform competition experiments with new probes and their target bacteria.

References:

- ⁱ Sinus and Allergy Health Partnership, Antimicrobial Treatment for Acute Bacterial Rhinosinusitis, *Otolaryngology-Head and Neck Surgery* July 2000: 123-1: S12. Figure 6
- ⁱⁱ Ludwig, W. Schleifer K.H. et. al. Phylogeny of bacteria beyond the 16S rRNA standard. *ASM News* 1999 65: 752-757
- ⁱⁱⁱ Trotha, R.; Hanck, T.; Konig, W.; Konig, B. Rapid ribosequencing – an effective diagnostic tool for detecting microbial infection" *Infection* 2001, 29: 12-16.
- ^{iv} Knut, R. Flateland S, Hanssen JF, Bengtsson G, Nissen, Hilde, Development and Evaluation of a 16S Ribosomal DNA Array-Based Approach for Describing Complex Microbial Communities in Ready-To-Eat Vegetable Salads Packed in a Modified Atmosphere. *Applied and Environmental Microbiology* 2002 :68 1146-1156
- ^v Perry-O'Keefe, H.; Rigby, S.; Oliveira, K.; Sorensen, D.; Stender, H.; Coull, J.; Hyldig-Nielsen, J. J. "Identification of indicator microorganisms using a standardized PNA FISH method" *J. Microbiol. Meth.* 2001, 47:281-292.
- ^{vi} Roland, P. S.; Stroman, D. W. "Microbiology of Acute Otitis Externa" *The Laryngoscope* 2002, 112, 1166-1177.
- ^{vii} Lu and Rothberg et. al. Reflective Interferometric Sensor for Label-Free Detection of Biomolecules (In Press)
- ^{viii} Chan, S.; Horner, S. R.; Miller, B. L.; Fauchet, P. M. "Identification of Gram Negative Bacteria using Nanoscale Silicon Microcavities" *J. Am. Chem. Soc.*, 2001, 123, 11797-11798.
- ^{ix} Chan, S.; Horner, S. R.; Miller, B. L.; Fauchet, P. M. "Identification of Gram Negative Bacteria using Nanoscale Silicon Microcavities" *J. Am. Chem. Soc.*, 2001, 123, 11797-11798.
- ^x Wilchek M, Bayer EA. Introduction to avidin-biotin technology. *Methods Enzymol.* 1990;184:5-13.
- ^{xi} Knut, Supranote 4
- ^{xii} Perry- O'Keefe H, Rigby S, Oliveira K, Sorensen D, Stender H, Coull J, Hyldig-Nielsen J, Identification of indicator microorganisms using standardized PNA FISH method, *Journal of Microbiological Methods*, 2001. 47: 281-292
- ^{xiii} <http://www.cat.cc.md.us/courses/bio141/labmanua/lab4/index.html>
- ^{xiv} Lu, J.; Strohsahl, C. M.; Miller, B. L.; Rothberg, L. J., in preparation.
- ^{xv} Mackie PL, Joannidis PA, Beattie J. Evaluation of an acute point-of-care system screening for respiratory syncytial virus infection. *J Hosp Infect.* 2001 May;48(1):66-71.
- ^{xvi} Giesecke KE, Mackenzie T, Roe MH, Todd JK Comparison of two rapid *Streptococcus pyogenes* diagnostic tests with a rigorous culture standard. *Pediatr Infect Dis J.* 2002 Oct;21(10):922-7.
- ^{xvii} Lai SY, Deffenderfer OF, Hanson W, Phillips MP, Thaler ER. Identification of upper respiratory bacterial pathogens with the electronic nose. *Laryngoscope.* 2002 Jun;112(6):975-9.
- ^{xviii} Versalovic J, Lupski JR. Molecular detection and genotyping of pathogens: more accurate and rapid answers. *Trends Microbiol.* 2002;10(10 Suppl):S15-21.

Each of the above-listed references is hereby incorporated by reference in its entirety.

In accordance with one aspect of the present invention, nucleic acid molecules are used as probes in accordance with a particular sensor device. The nucleic acid molecules can be identified from genomic databases for the particular organism (pathogens) to be identified. It is preferred to utilize DNA molecules that are highly selective; that is, the DNA molecule will hybridize to only the target nucleic acid molecule of a single pathogen. Thus, the probe and target nucleic acid molecule should represent unique complementary sequences. The target sequence and, thus, the probe sequence can be identified by screening genomic databases of an organism.

Exemplary pathogens whose nucleic acid molecules can be detected in accordance with the present invention include, without limitation, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, α and β hemolytic *Streptococcus*, *Pseudomonas aeruginosa*, parainfluenzae viruses, influenzae viruses, rhinoviruses, as well as fungi and parasites that are known to cause ear-nose-throat infections.

Pathogen target nucleic acid molecules can be DNA or RNA (i.e., rRNA or mRNA). A preferred target nucleic acid molecule is rRNA. rRNA target nucleic acids can be identified from existing or later-modified electronic databases, such as (1) the European ribosomal RNA database, which compiles all complete or nearly complete SSU (small subunit) and LSU (large subunit) ribosomal RNA sequences; and (2) the Ribosomal Database Project (operated at Michigan State University); and (3) the 5S rRNA Database (Szymanski et al., *Nucleic Acids Research* 28(1):166-167 (2000), which is hereby incorporated by reference in its entirety).

The probe nucleic acid molecule, whether DNA or RNA, can be bound to the sensor device in accordance with known procedures. These are described, for example, in the following patent applications describing particular types of sensors:

nanocrystal based sensors of the type disclosed in U.S. Patent Application Serial No. 10/171,136 to Miller et al., filed June 13, 2002, which is hereby incorporated by reference in its entirety;

interferometric sensors of the type disclosed in U.S. Patent Application Serial No. 10/082,634 to Chan et al., filed February 21, 2002, which is hereby incorporated by reference in its entirety;

reflective interferometric sensors of the type disclosed in U.S. Patent Application Serial No. 10/282,274 to Miller et al., filed October 28, 2002, which is hereby incorporated by reference in its entirety; and

nucleic acid hairpin fluorescent sensors of the type disclosed in U.S. Provisional Patent Application Serial No. 60/437,780 to Miller et al., filed January 2, 2003, which is hereby incorporated by reference in its entirety.

Sensors of the above-identified applications can be readily modified to incorporate nucleic acid probes of the present invention that are substantially complementary to particular nucleic acid targets. Detection of the hybridized target is described in the above-identified applications.

As used in the following claims, "biological sample" can refer to a biological sample obtained directly from a patient, whether in the form of a swab culture sample or a body fluid, or a biological sample that is cultured for a period of time, i.e., in a culture medium.

Hybridization, as between any target nucleic acid in the biological sample and the one or more nucleic acid probes bound to the sensor device substrate, can be achieved in any suitable hybridization medium. The stringency of the hybridization conditions can be modified in accordance with known procedures. Typically, however, the hybridization will be under stringent conditions so as to minimize the likelihood of non-specific binding between the non-target nucleic acid molecules in the biological sample and the one or more probes. Stringency of hybridization can be increased by increasing the temperature at which hybridization and/or wash occurs, as well as by minimizing the salt content of the hybridization medium or the wash medium. Typically, the wash conditions are at about the same or greater stringency than the hybridization conditions. Details concerning hybridization protocols are known in the art and described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory (1989).

Although a preferred application of the present invention concerns the method of detecting the presence of an otolaryngologic pathogen in a biological sample, as well as sensor devices capable of use in performing such a method, as noted elsewhere in the present application, the present invention also relates to other applications. These include, among others, methods of detecting the presence of the otolaryngologic pathogen *Streptococcus pneumoniae* via hyaluronate lyase activity on substrate-bound hyaluronic acid disaccharide; detection of bacteria, viruses, fungi, or any host of allergic diseases via substrate-bound antibodies specific for the bacteria, virus, fungus, or allergen; detection of cellular morphology using substrate-bound DNA or antibody directed to, e.g., p53, Bcl-2, Cyclin D1, c-myc, p21ras, c-erb B2, or CK-19; detection of acid reflux disease via substrate-bound polypeptides that are substrates for pepsin; and detection of B-2 transferrin or other molecules that are sensitive to the diagnosis of cerebrospinal fluid leaks.

Background

- Point of Care Diagnostics in High Demand



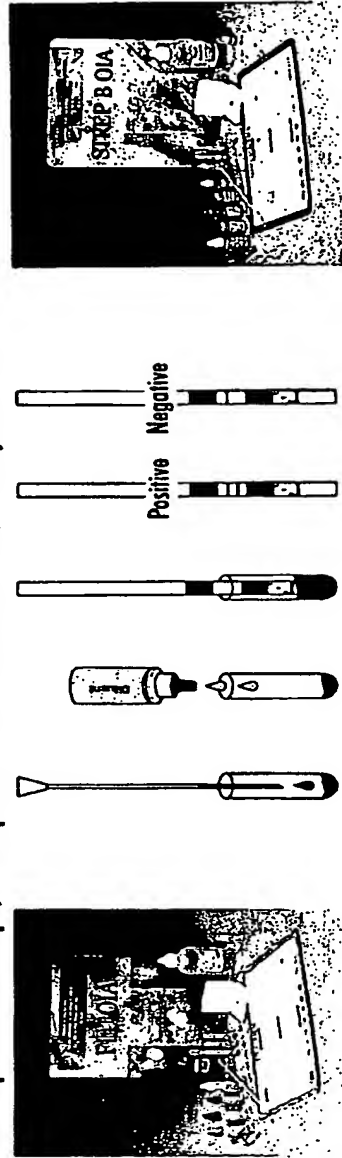
- Major Public Health Implications For Rapid Detection Techniques

Background

- Advantages of Point of Care Testing
 - Reduce Infection Transmission
 - Reduce Antibiotic Use
 - Reduce Cost of Care
 - Allow Better remote patient monitoring/assessment

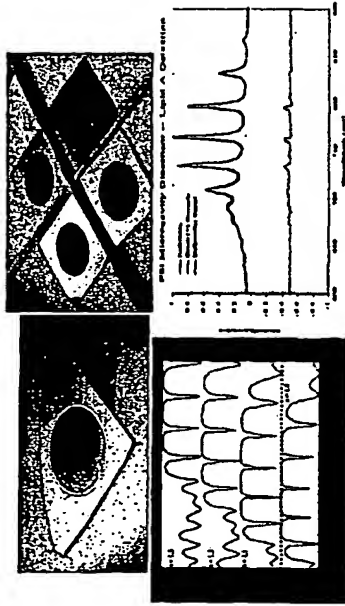
• Limited Rapid Tests Available Today

- Rapid Strep, Rapid Infl A and B, Rapid Mono, RSV, bHcg



Collaboration

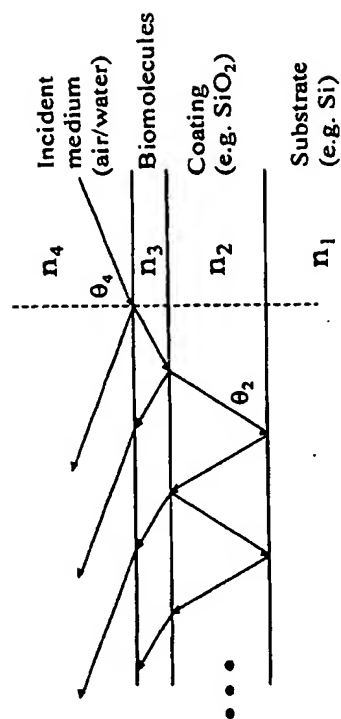
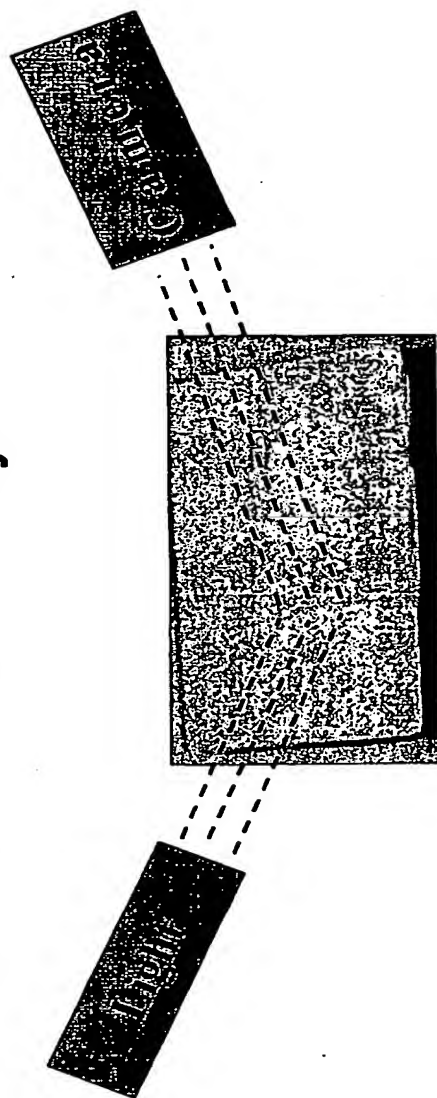
- Center for Future Health, Pathogen Detection Laboratory



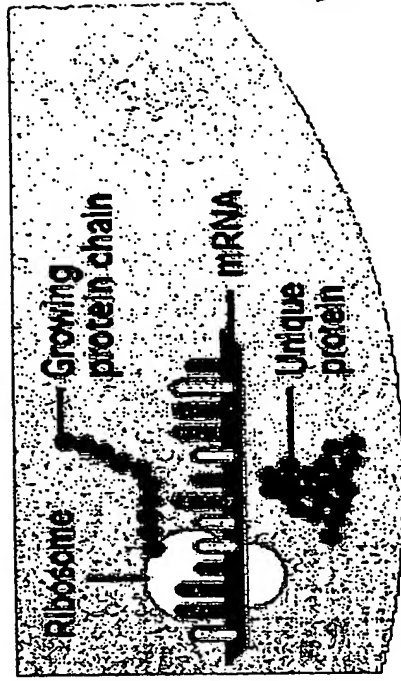
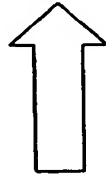
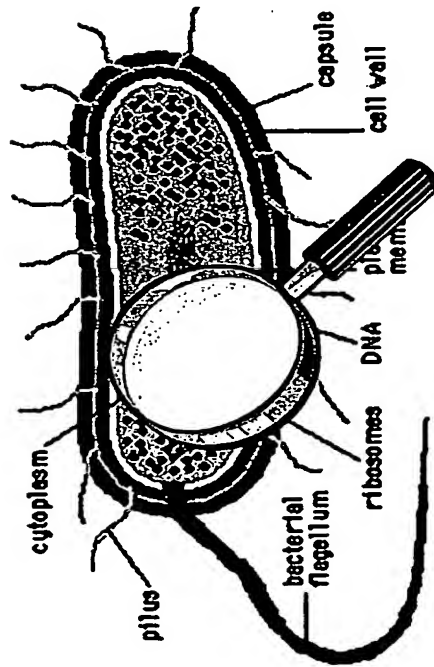
- Collaboration started
- Research Conducted

The Sensor

- Silicon Wafers - 150 nm layer of thermal oxide



The Theory



-22-

- Probes – Small Sequences to attach rRNA



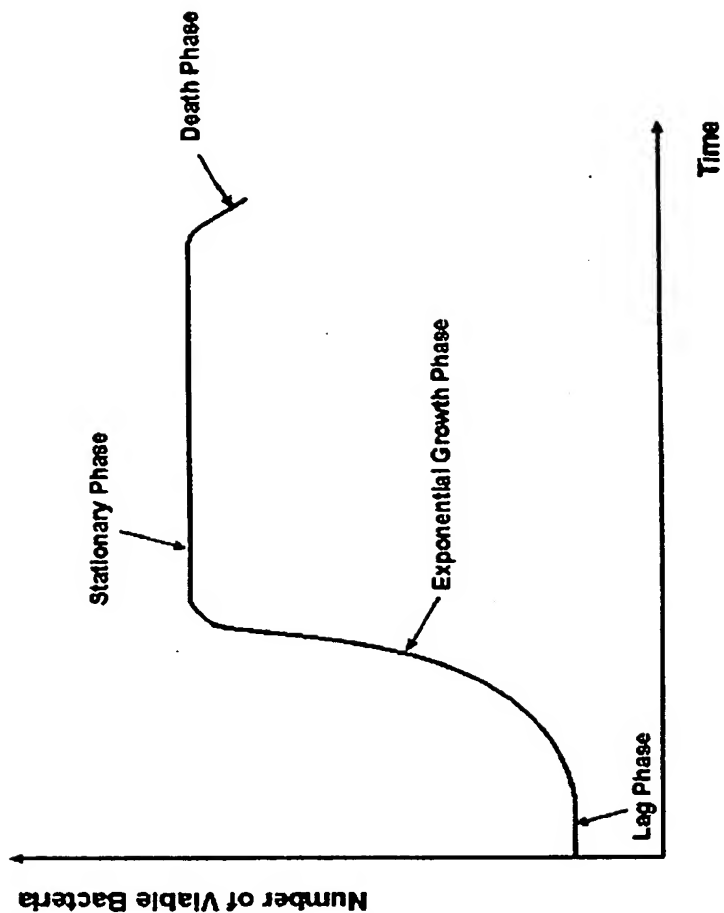
Probe



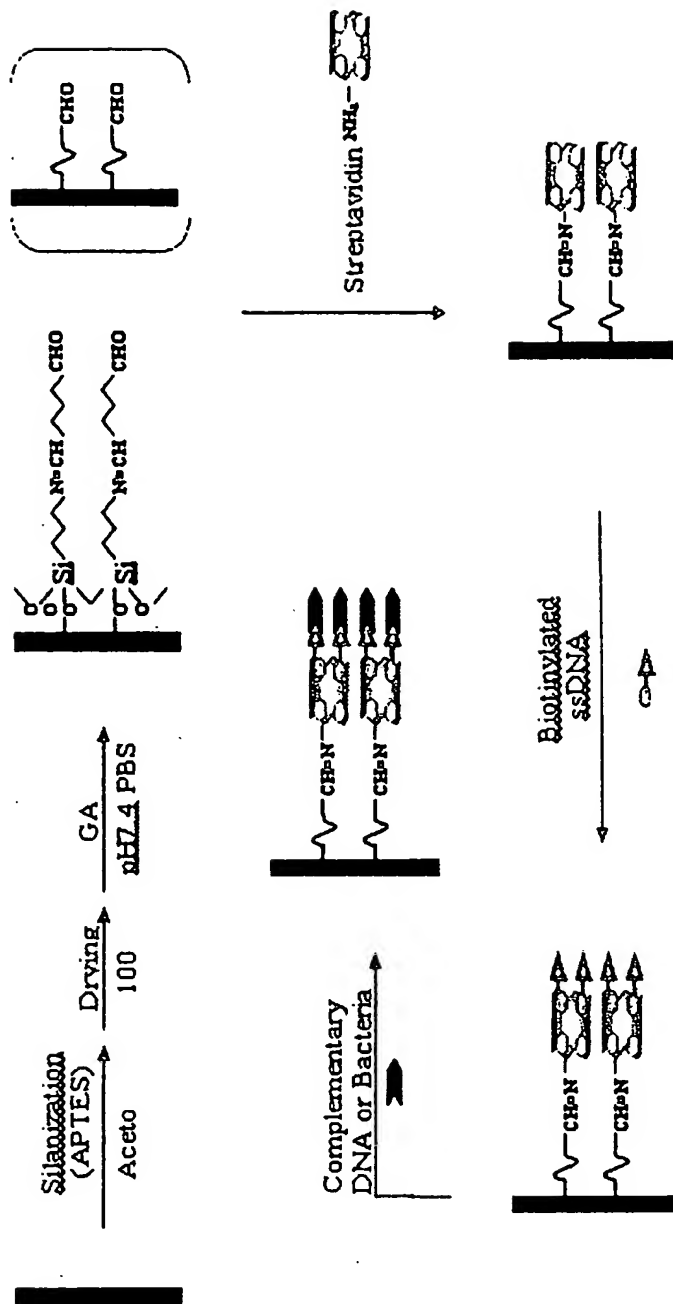
Bacteria RNA

The Theory

- 23 -



The Surface Chemistry



• *Pseudomonas* Chosen Based on Probe Selectivity

Probe 1 - Biotin-CCT-TGC-GCT-ATC-AGA-TGA-GCC-TAG-GT

Journal of Microbiological Methods, 2001. 47: 281-292

Probe 2 - Biotin-CTG-AAT-CCA-GGA-GCA

Applied and Environmental Microbiology 2002 :68 1146-1156

Experiments

- DNA Experiments

.2 nm Change Should Detect 10% of 15 base oligonucleotide



Bacteria RNA



Probe

- Data on this visualization in Press Currently

Experiments

• Bacterial Experiments

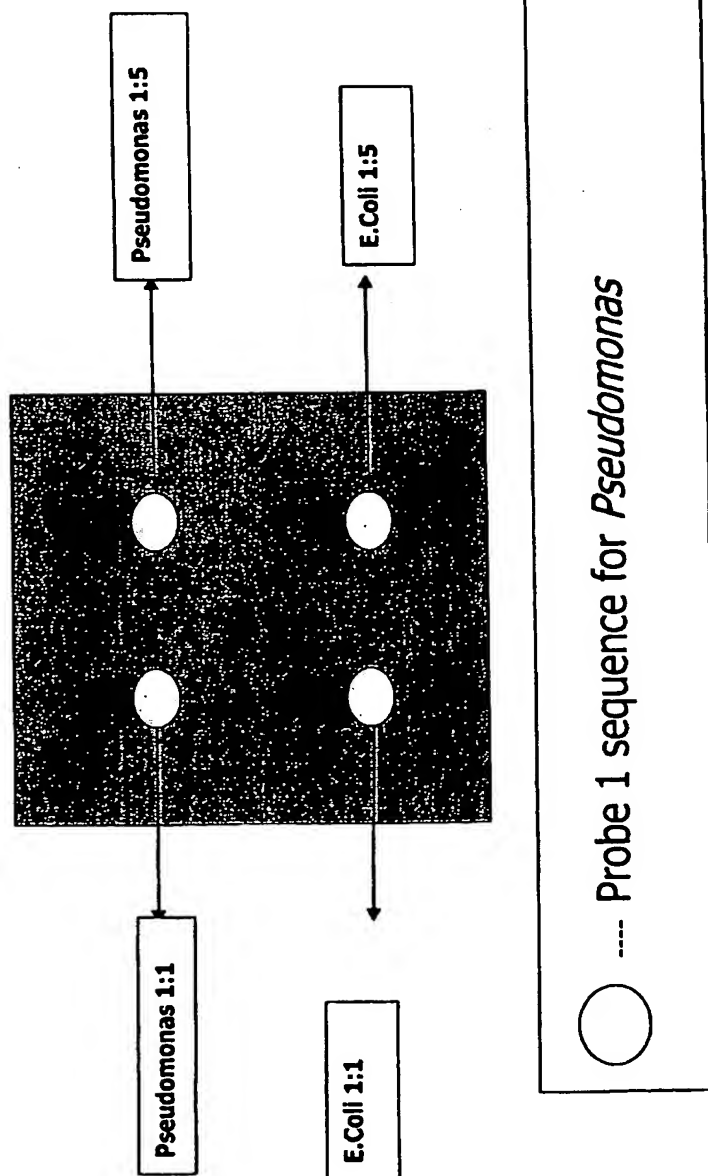


Bacteria rRNA

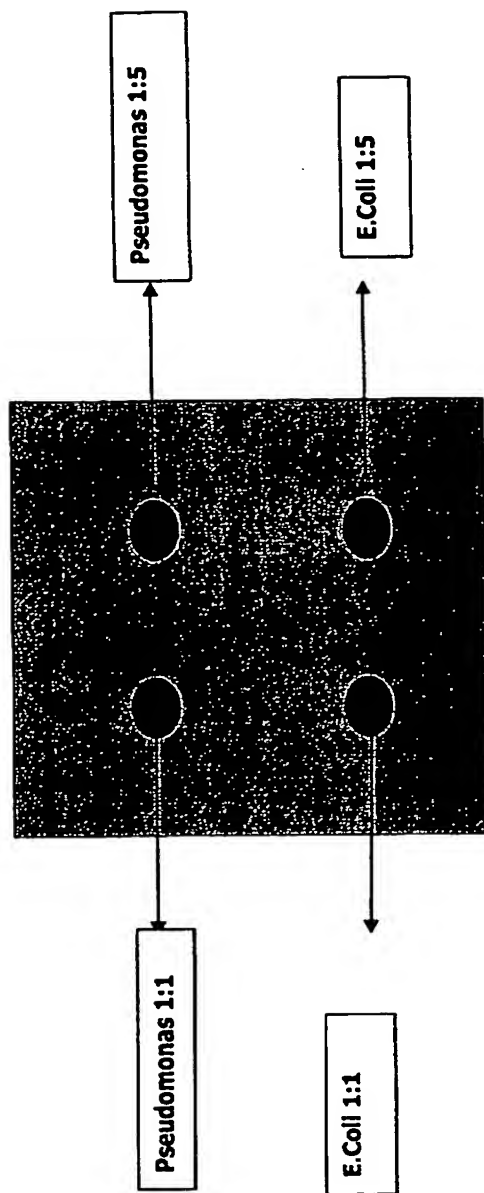
Probe

• Experiment 1 - Bacteria Centrifuged. Concentrated Pellet Resuspended in PBS

Experiment 1 Set-Up

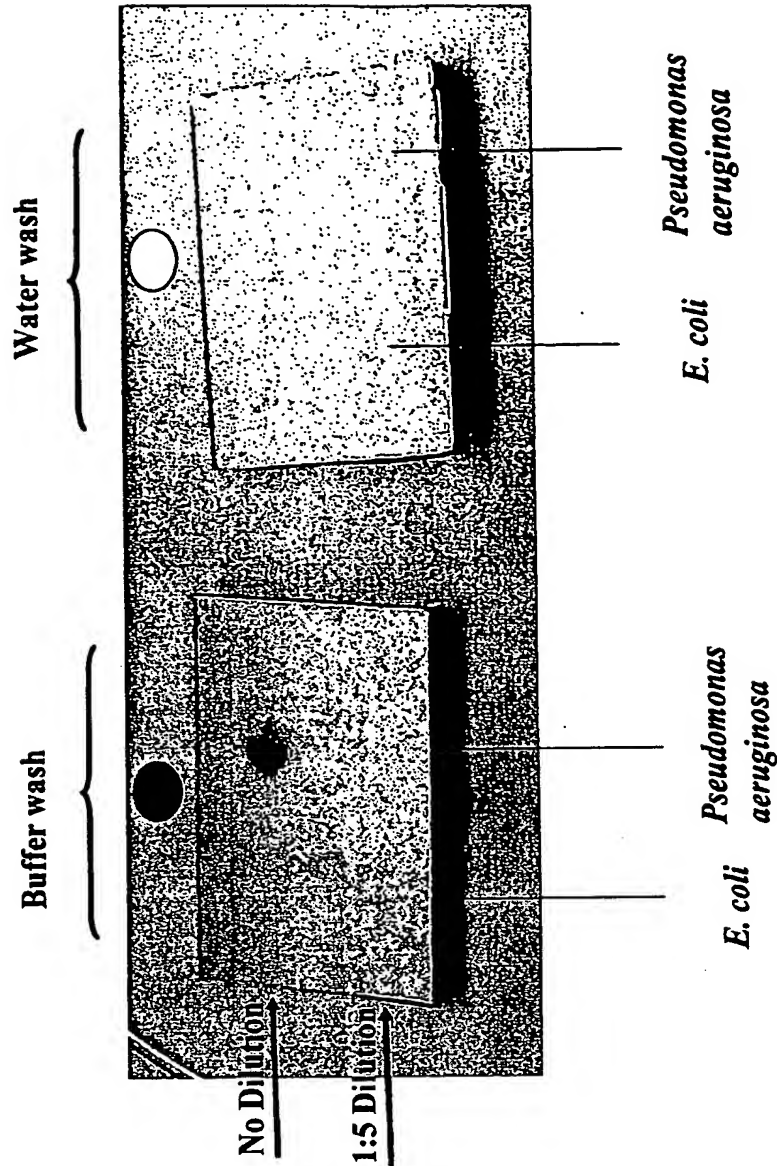
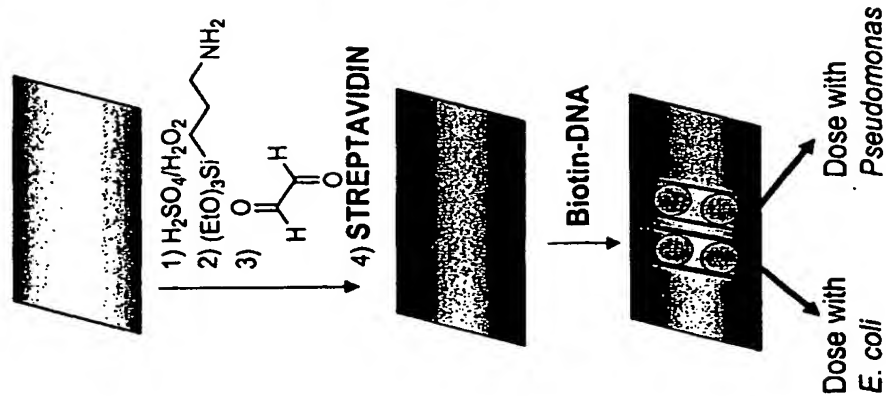


Experiment 1 Set-Up

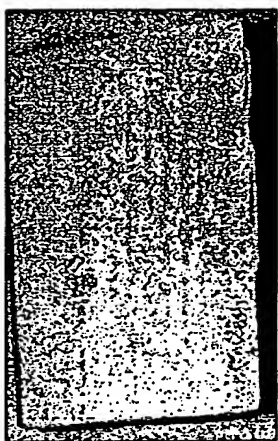


● --- Probe 2 sequence for *Pseudomonas*

Experiment 1 Results

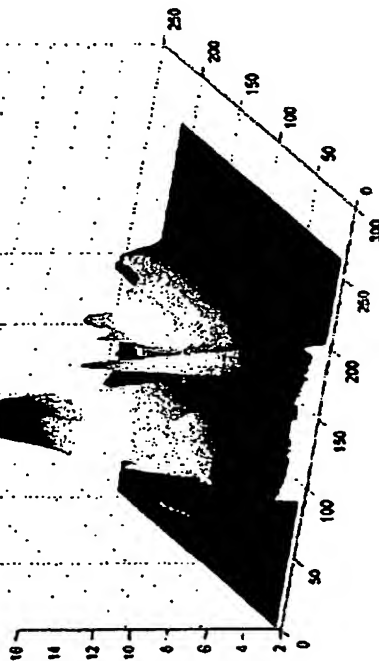


Experiment 1 Results

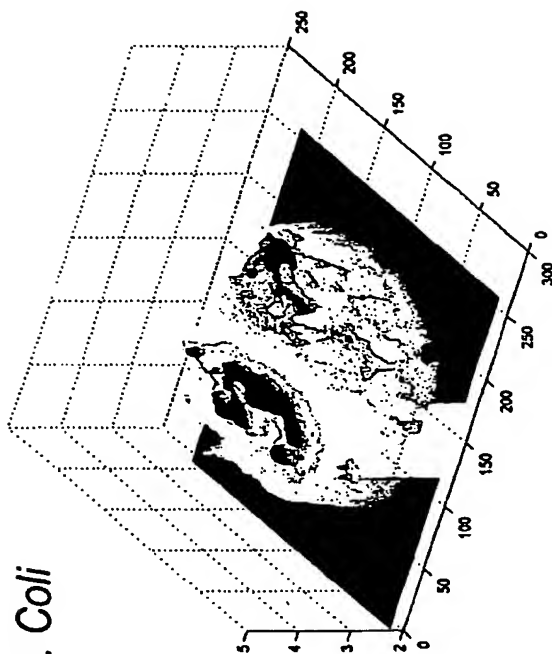


After H2O Rinse

Pseudomonas

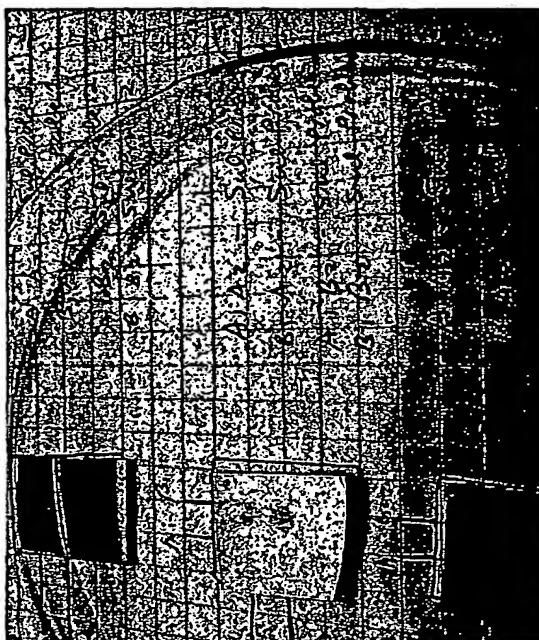


E. Coli

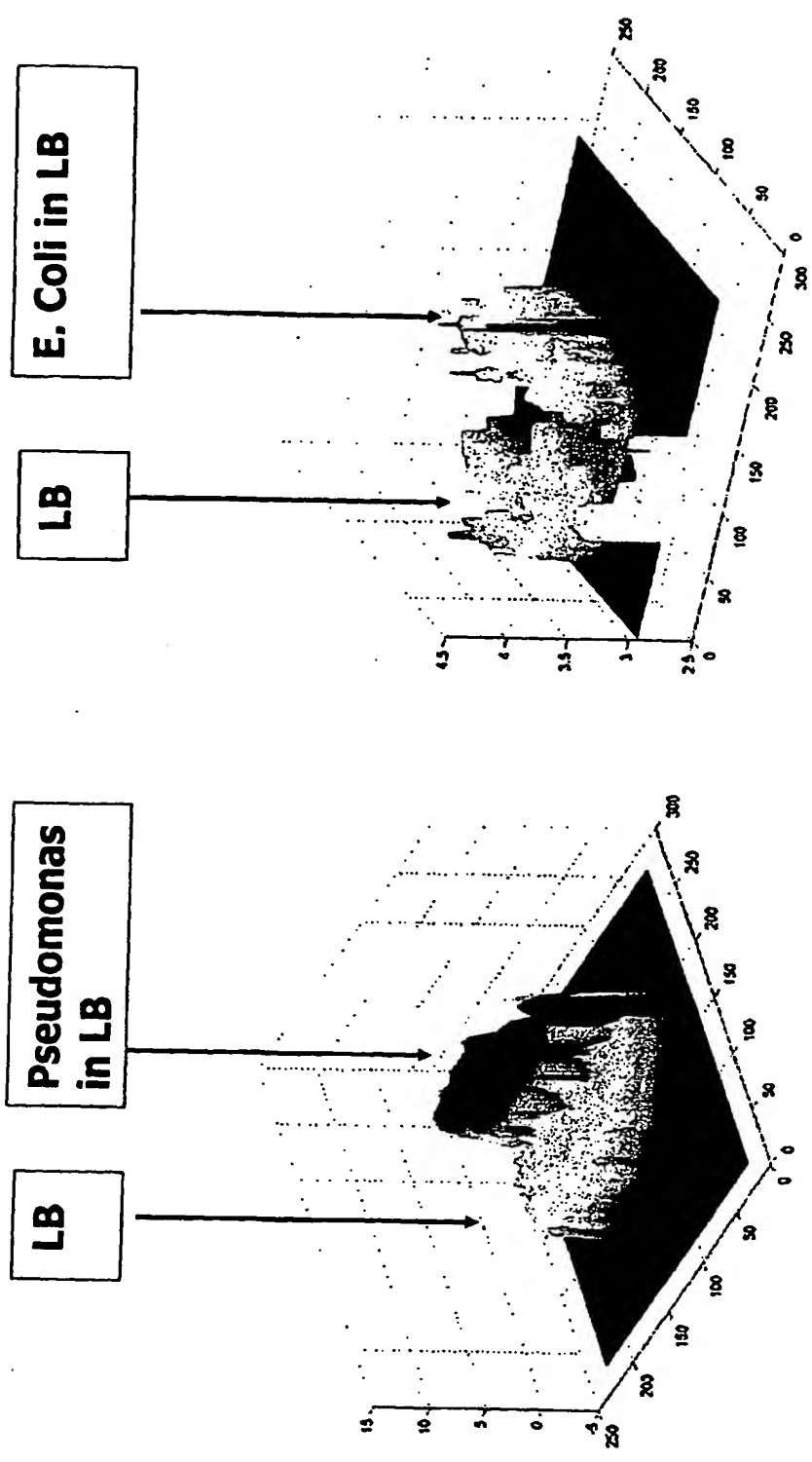


Experiment 2 Set Up

- Bacteria Taken Directly out of overnight LB Culture and Placed on Chip
- LB Media and *E. Coli* in LB Run as control

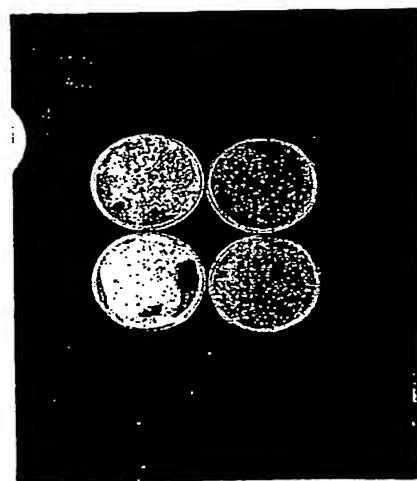
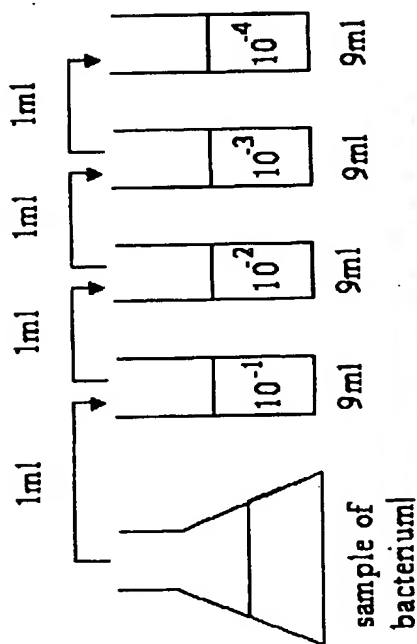


Experiment 2 Results



Experiment 3 Set-Up

- How few bacteria can this method detect?
- Use standard fresh bacterial dilutions and decipher detection sensitivity.



Sample No. 101

PROJECT NO. 101

BOOK NO.

A₁ A₂ - Probe 1 5ml Plate 1000

B₁ B₂ - Probe 1 5ml Plate 1000

A₁ B₁ - Probe 2 5ml Plate 1000

B₁ B₂ - Probe 2 5ml Plate 1000

A₁ A₂ - Probe 3 1/100 Plate 1000

B₁ A₂ - Probe 3 1/100 Plate 1000

A₁ B₁ - Probe 3 1/100 Plate 1000

B₁ B₂ - Probe 3 1/100 Plate 1000

A₁ A₂ - Probe 4 1/1000 Plate 1000

B₁ A₂ - Probe 4 1/1000 Plate 1000

A₁ B₁ - Probe 4 1/1000 Plate 1000

B₁ B₂ - Probe 4 1/1000 Plate 1000

A₁ A₂ - Probe 5 1/10000 Plate 1000

B₁ A₂ - Probe 5 1/10000 Plate 1000

A₁ B₁ - Probe 5 1/10000 Plate 1000

B₁ B₂ - Probe 5 1/10000 Plate 1000

A₁ A₂ - Probe 6 1/100000 Plate 1000

B₁ A₂ - Probe 6 1/100000 Plate 1000

A₁ B₁ - Probe 6 1/100000 Plate 1000

B₁ B₂ - Probe 6 1/100000 Plate 1000

A₁ A₂ - Probe 7 1/1000000 Plate 1000

B₁ A₂ - Probe 7 1/1000000 Plate 1000

A₁ B₁ - Probe 7 1/1000000 Plate 1000

B₁ B₂ - Probe 7 1/1000000 Plate 1000

A₁ A₂ - Probe 8 1/10000000 Plate 1000

B₁ A₂ - Probe 8 1/10000000 Plate 1000

A₁ B₁ - Probe 8 1/10000000 Plate 1000

B₁ B₂ - Probe 8 1/10000000 Plate 1000

A₁ A₂ - Probe 9 1/100000000 Plate 1000

B₁ A₂ - Probe 9 1/100000000 Plate 1000

A₁ B₁ - Probe 9 1/100000000 Plate 1000

B₁ B₂ - Probe 9 1/100000000 Plate 1000

A₁ A₂ - Probe 10 1/1000000000 Plate 1000

B₁ A₂ - Probe 10 1/1000000000 Plate 1000

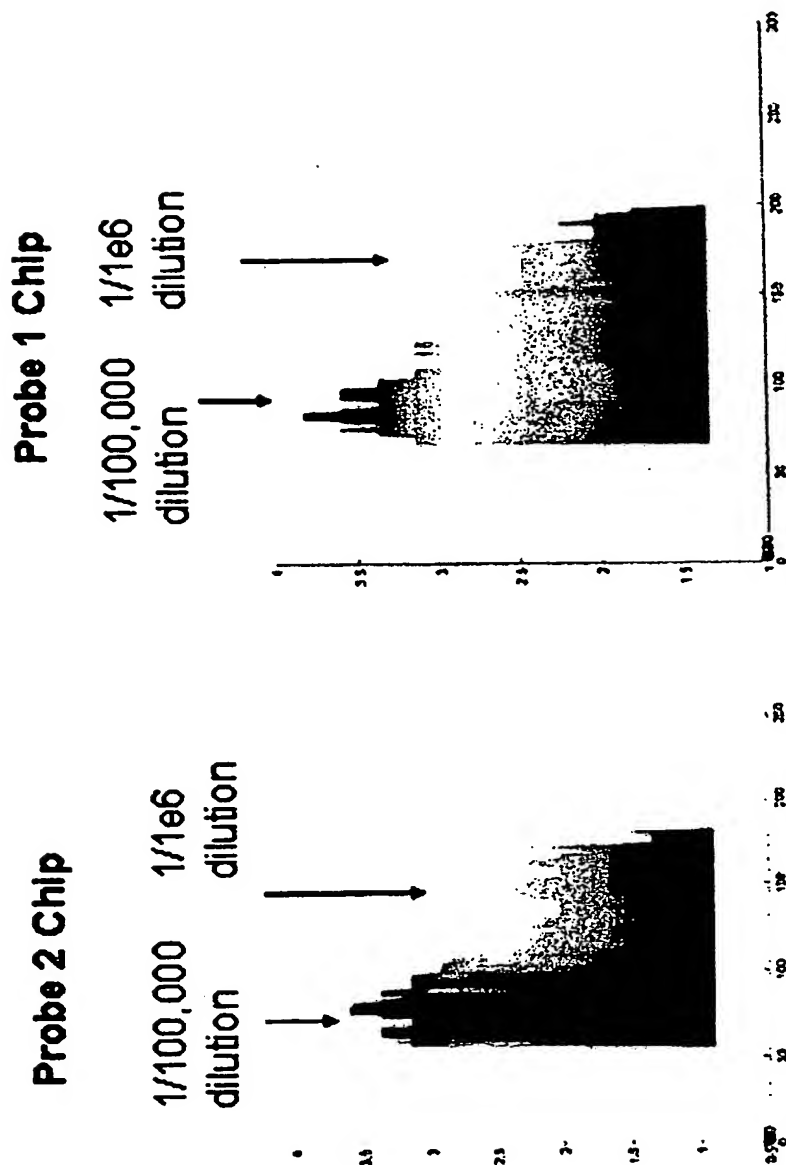
A₁ B₁ - Probe 10 1/1000000000 Plate 1000

B₁ B₂ - Probe 10 1/1000000000 Plate 1000

Next continued to Page

Experiments 3 Results

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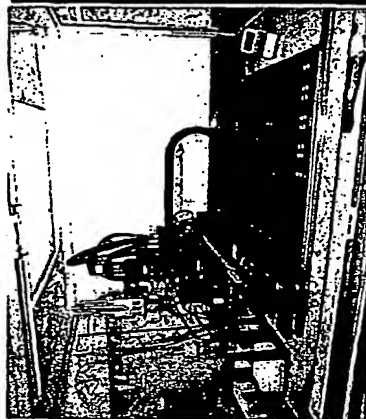
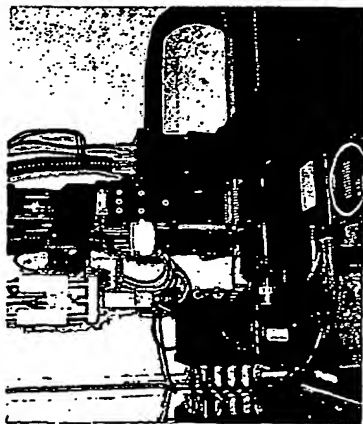
- White light reflective limit 125 and 160 CFU/spot detection

Current Work

- Improving the reflective technique
 - Moving to laser sources for the light
 - Improving Analysis Software
 - Shortening Chip analysis time
- Chip Issues
 - Finding rinse solutions that minimize residue
- Standardizing data between different chips
 - Finding new ways to store chips for long term use
 - Finding better ways to spot probes on chips

Current Work

- Use of microarrays to get probes on chips



- Probe Selection

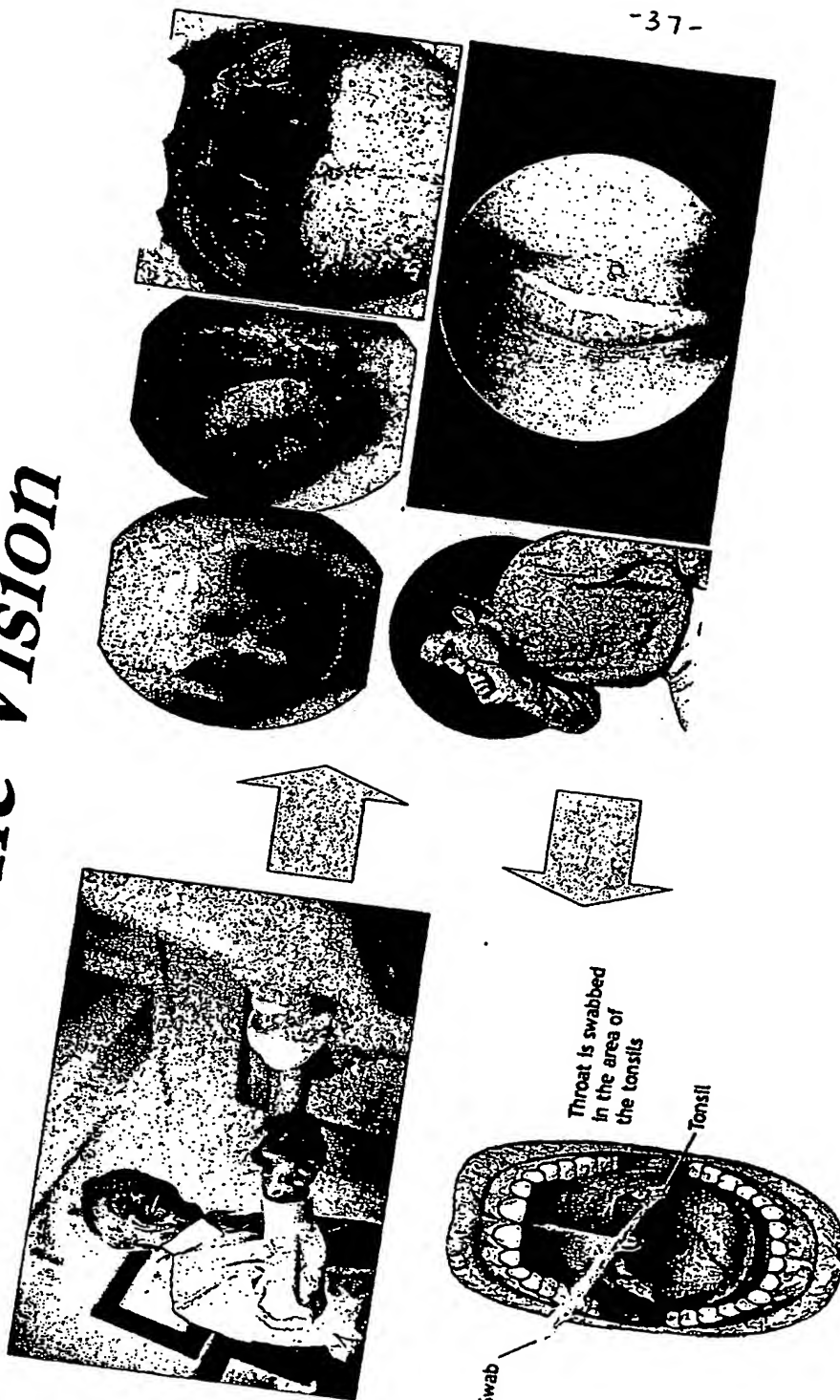
- DNA libraries of many bacteria available

- Clinical Experiments

- Testing chips on human samples

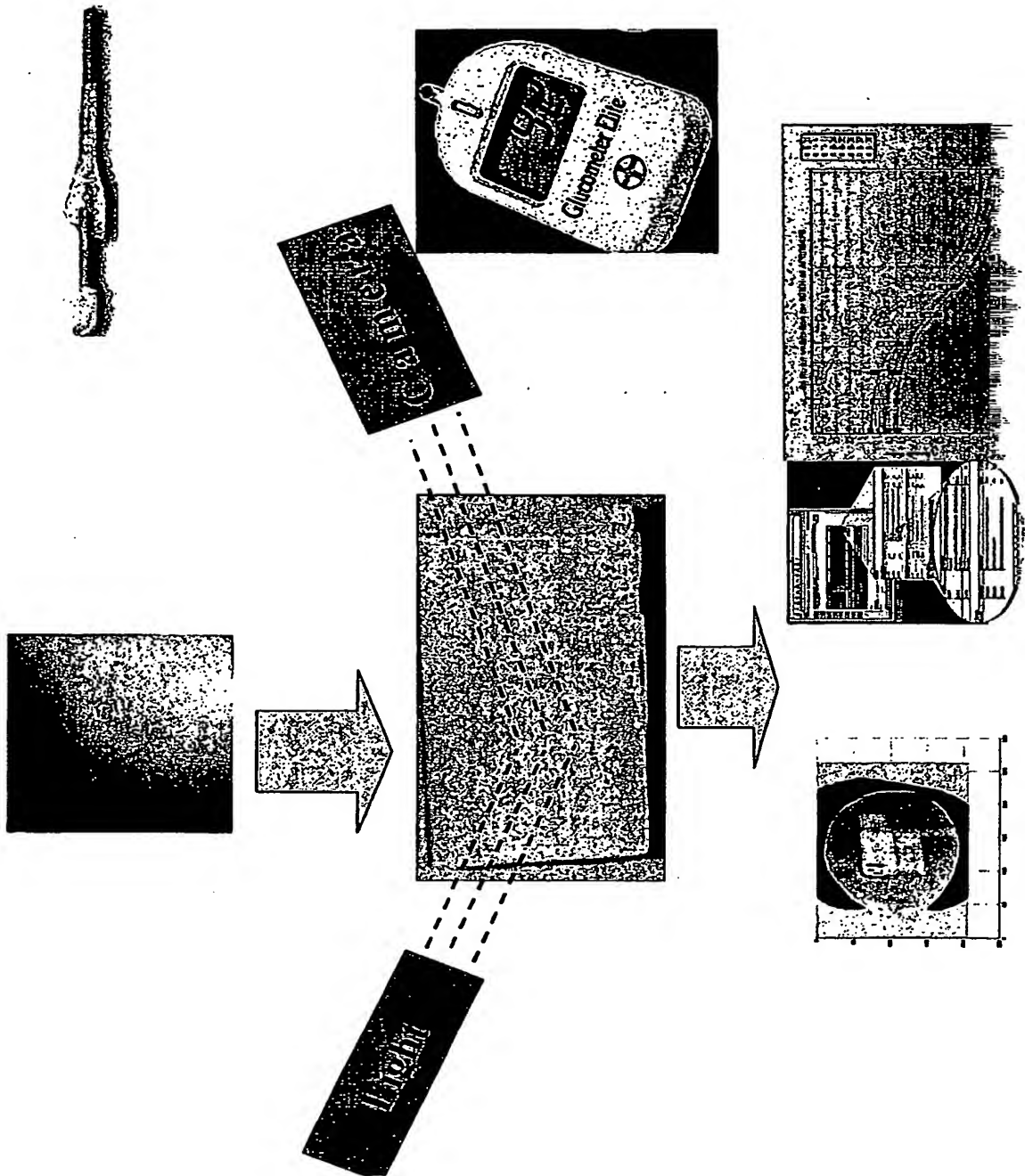
The Vision

-37-



©ADAM

The Vision



What is claimed:

1. A method of detecting the presence of an otolaryngologic pathogen in a biological sample:
 - providing a sensor device comprising (i) a substrate to which has been bound one or more nucleic acid probes, and (ii) means for detecting the binding of a target nucleic acid to the one or more nucleic acid probes, the target nucleic acid being specific to an otolaryngologic pathogen;
 - exposing a biological sample, or a portion thereof, to the sensor device under conditions effective to allow hybridization between the one or more nucleic acid probes and the target nucleic acid to occur; and
 - detecting, with said means for detecting, whether any target nucleic acid molecule hybridizes to the one or more nucleic acid probes, wherein hybridization indicates the presence of the otolaryngologic pathogen in the biological sample.
2. The method according to claim 1 wherein the otolaryngologic pathogen is selected from the group consisting of *Haemophilus influenzae*, *Streptococcus pneumonia*, *Moraxella catarrhalis*, α and β hemolytic *Streptococcus*, *Pseudomonas aeruginosa*, parainfluenzae viruses, influenzae viruses, rhinoviruses, fungi, and parasites.
3. The method according to claim 1 wherein the target nucleic acid is an RNA molecule.
4. The method according to claim 1 wherein the target nucleic acid is an rRNA molecule.
5. A sensor device comprising:
 - a substrate to which has been bound one or more nucleic acid probes, and
 - means for detecting the binding of a target nucleic acid to the one or more nucleic acid probes, the target nucleic acid being specific to an otolaryngologic pathogen selected from the group consisting of *Haemophilus influenzae*, *Streptococcus pneumonia*, *Moraxella catarrhalis*, α and β hemolytic *Streptococcus*, *Pseudomonas aeruginosa*, parainfluenzae viruses, influenzae viruses, rhinoviruses, fungi, and parasites.
6. The sensor device according to claim 5 wherein the target nucleic acid is an RNA molecule.
7. The sensor device according to claim 5 wherein the target nucleic acid is an rRNA molecule.

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